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Water-to-Air Transfer and Enrichment of Bacteria in Drops from Bursting Bubbles

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An electrostatic induction technique was used to determine both drop size distribution and concentration of bacteria in the film drops produced by bubbles bursting at the surface of a suspension of *Serratia marcescens*. Film drops are produced from the collapse of the thin film of water that just before bursting separates the air in the bubble from the atmosphere. Bubbles of 1.7-mm diameter produced from 10 to 20 film drops which ranged from <2 μm to over 30 μm in diameter. Half the drops were <10 μm. For bubbles rising a distance of less than 2 cm through the bacterial suspension, bacterial enrichment factors in the drops were between 10 and 20. Electrostatic methods can be used to determine the enrichment of bacteria in film drops as a function of bubble size and distance of rise through the bacterial suspension.

Most of the material ejected into the atmosphere from freshwaters and the sea is thought to come from the bursting of air bubbles (3, 13). Produced in great quantity by breaking waves and precipitation (9, 10), bubbles rise to and burst at the surface of the water to produce both film and jet drops (Fig. 1). The film drops, generated when the bubble film collapses, have a size distribution that can cover over two orders of magnitude: from less than 0.1 to over 10 μm in diameter (3, 10). However, jet drops, produced from the jet that rises from the collapsing bubble cavity, seldom vary in size by more than a factor of two or three. Their diameter is about one-tenth that of the bubble.

Bacteria can be highly enriched in jet drops from bubbles that burst at the surface of bacterial suspensions (1, 2, 8, 15). Enrichment factors (EFs; the ratio of the concentration of bacteria in the drop to that in the bulk suspension) vary, depending upon a number of reasons, from 1 to more than 1,000. EFs for bacteria in film drops are not known. R. J. Cipriano (Ph.D. thesis, State University of New York at Albany, 1979) measured EFs of 50 to 100 in drops smaller than 10 μm in diameter. Cipriano inferred these to be film drops, since both jet and film drops were collected. Although there were several good reasons for making this inference, it is imperative that direct observations be made of the bacterial EFs in drops that are unequivocally known to be film drops.

The number of jet drops decreases with bubble size, but that of film drops increases (3, 11). Bubbles of less than 0.3-mm diameter produce five or more jet drops, whereas those of 6 mm produce only one. However, although there is no film drop production for 0.3-mm bubbles, a maximum of about 1,000 film drops are produced by a 6-mm bubble. Clearly, the shape of the bubble spectrum determines which drop predominates in the aerosol produced at the surface of the water. Recent work (10) on bubble spectra strongly suggests, contrary to earlier work, that drops of <5 or 10 μm in diameter are mostly film drops.

In view of this, plus the lack of direct observations of the bacterial EF in film drops, the present work was done. An additional motivation is that many film drops are in the size range of 1 to 10 μm, a size that allows penetration into the lungs (14). This is an important reason for determining the health hazards of pathogens in airborne drops.

MATERIALS AND METHODS

Preparation of the bacterial suspension. In each of three experiments, done several weeks apart, the cells of a 20-h culture of *Serratia marcescens* in Difco nutrient broth were spun down in a centrifuge and washed twice. In the first experiment, the cells were added to unsterilized pond water and mixed on a magnetic stirrer to produce a concentration of 2.3×10^6 ml^{-1}. In the second, the cells were added to pond water and diluted six times with distilled water to a cell concentration of 5.4×10^5 ml^{-1}. In the final experiment, the washed cells were added directly to distilled water and mixed on a magnetic stirrer. The cell concentration was 3.4×10^5 ml^{-1}. Bulk concentrations were obtained by serial dilutions at the beginning of, during, and at the end of each experiment. The experiments were done at a room temperature of about 22°C.

Production of film drops. Film drops were produced from air bubbles bursting individually at the surface of

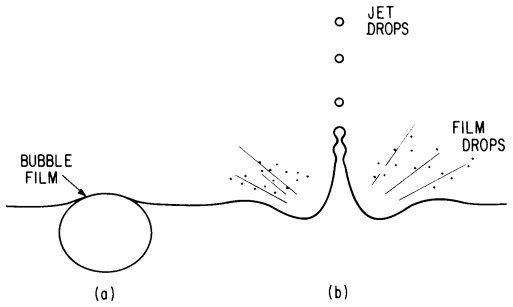

FIG. 1. (a) Bubble at rest at the surface; (b) jet and film drops produced by a bursting bubble.

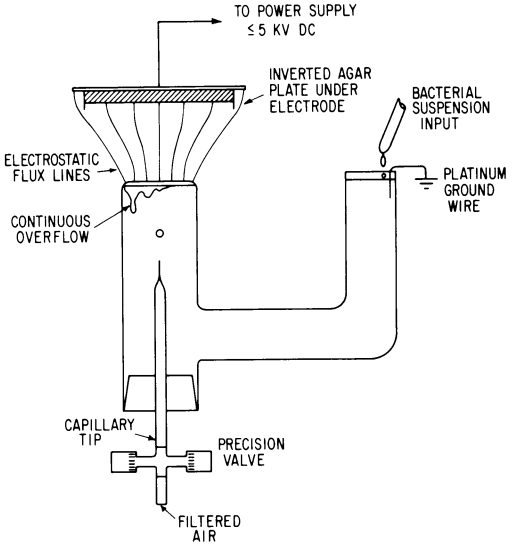

FIG. 2. Method by which electrostatic induction causes film drops from bursting bubbles to move upward and strike whatever is attached to the bottom of the electrode. Shown here is an agar plate.

the bulk suspension in the apparatus shown in Fig. 2. The main section of the two-branched glass device was a tube of 3 cm inside diameter and 12 cm long. The bacterial suspension was allowed to drip slowly into the right-hand branch from a small reservoir placed well above the experiment. At the rate of 1 to 2 drops per s, this produced a continuous overflow of the surface where the bubbles burst. The buildup of compressed surface-active monolayers was thus prevented and a clean, reproducible surface was available for each bubble.

Bubbles were produced by forcing filtered air through a glass capillary tip inserted through a polyethylene stopper. We have described in detail elsewhere how to make a capillary tip to produce a bubble of any desired size (6). The tip, which was used for all experiments, produced a bubble 1.7 mm in diameter. Although, as mentioned earlier, larger bubbles produce more film drops, these bubbles are not produced in nature in such numbers as are smaller bubbles. Recent work suggests that bubbles in the size range of 1 to 3 mm are most important for film drop production (10).

Since bubbles sometimes stick at the surface for a few seconds before bursting, it was necessary to produce them individually when desired. We accomplished this with a precision metering valve placed in the air line as close as possible to the capillary tip. For individual, on-demand bubble production, it is vital to keep the air volume between the valve and the capillary tip as small as possible. This is especially true for the larger bubbles. In lieu of a precision valve and a compressed-air line, bubbles can be produced as required with a syringe and two simple screw clamps (6).

The bubble rise distance (BRD; the distance from the capillary tip to the water surface) is easily adjusted by sliding the capillary tip up or down through the hole in the stopper.

Collection of film drops. Calculation of the bacterial EF for the drops requires a knowledge of both the total volume of the film drops and the number of bacteria they carry. This was obtained by an electrostatic induction technique. Since some of this has been described elsewhere (5), only a brief outline follows.

If a bubble bursts in the presence of a sufficiently strong electric field, both film drops and jet drops acquire an induced charge. This charge produces an upward-directed electric force that overcomes gravity, and the drops move quickly toward the upper electrode. They can be collected on anything attached to the bottom of the electrode. In this work the drops were collected on a gelatin-coated glass slide (5 cm square) taped to the center of a metal electrode (9 by 15 cm). The electrode was placed 1 cm above the water surface and a negative potential of 1,000 V was applied to it. The water was grounded with a fine platinum wire. To insure an adequate collection of film drops, we allowed 50 or more bubbles to burst. A microscope was used to determine the diameter of the spots made by the drops in the gelatin. After application of a correction factor relating gelatin spot size to true drop size, the film drop size distribution was obtained.

We determined the number of viable bacteria carried by the drops by collecting the drops on inverted nutrient agar plates taped to the electrode (see Fig. 2) and counting the colonies that developed after about 20 h of incubation at room temperature. Similar to the experiment to determine drop size distribution, the potential gradient for the electrostatic collection was 1,000 V cm^{-1}. However, the electrode was 2 cm above the water with a negative potential of 2,000 V. The increased distance allowed the film drops to separate more horizontally before they hit the agar surface. Although two film drops separated by only a few micrometers on the gelatin slide could be detected easily, they had to be a few hundred micrometers apart for discrimination of the two colonies they might produce on the agar plate. This condition was easily satisfied. The average distance between the film drop colonies was 1 cm or more.

There was no possibility of confusing the film drop colonies with the single colony formed by the jet drops. Since the jet drops from the bubbles all moved directly upward to strike the agar at the same spot, a single large colony of 2 to 3 mm was produced. The

film drops, flung outward when a bubble burst, produced small colonies randomly distributed over the 9-cm diameter agar surface.

The EF for the film drops was obtained by dividing the number of colonies produced by the drops from a given number of bubbles by the volume of the drops, and then dividing by the bulk concentration. This gives the average EF. The nature of the variation between EF and drop size is unclear.

The major assumption made in calculating the EFs is that the drops producing the colonies on the agar plates contained only one bacterium. Clearly, if more than one colony-forming bacterium were in the drops, the actual EF would be more than that calculated. It is highly unlikely that more than one bacterium was carried by any of the drops, for only 1 drop in 93 produced a colony. The presence of a single cell in a drop produces a very large EF. Assuming one cell per drop, and working with a suspension of 3.4×10^5 ml^{-1} (as was used in Fig. 3), drops of 5, 10, and 30 μm would have EFs of 44,000, 5,600, and 210, respectively.

RESULTS

Since the results of the three experiments were similar, only the last of the three, which was more involved, will be presented and discussed in detail. In the first two experiments, the BRD was 0.65 cm. The experiment with the pond water suspension of *S. marcescens* (2.3×10^6 ml^{-1}) produced an average of about 12 film drops per bubble (calculated from an observed 597 drops per 50 bubbles), and the EF was about 14. The experiment with the six-times-diluted pond water suspension (5.4×10^5 ml^{-1}) produced an average of about 14 drops per bubble (we observed 703 drops from 50 bubbles), and the EF was about 11. In both experiments, the drop size distribution showed a maximum concentration at about 5 μm diameter. It decreased rapidly with increasing drop size, with a long tail extending out to about 35 μm.

If the bubbles do not burst immediately upon arrival at the surface, drop production is effectively eliminated. During part of one of the experiments the bubbles stuck momentarily at the surface before bursting. We exposed a gelatin slide to 50 such bubbles and collected only one film drop.

The final experiment (distilled water suspension of 3.4×10^5 ml^{-1}) was done at two BRDs, 0.9 cm and 1.9 cm. At each depth we exposed gelatin slides to 50 bursting bubbles and collected 990 and 501 drops from the 0.9-cm and 1.9-cm depths, respectively. This amounted to 19.8 drops per bubble burst at 0.9 cm and 10 drops at 1.9 cm depth. The drop size distribution is shown in Fig. 3 for both depths.

We attempted to expose 22 agar plates for each depth, and each plate to 50 bubbles that burst immediately. We succeeded only at the 0.9-cm depth, where 233 colonies were produced by the film drops from 1,100 bubbles. The average colony count per plate was 10.6, with a standard deviation of 3.9. At the 1.9-cm depth, many bubbles did not burst immediately. Thus, the plates were exposed to only 672 bubble bursts, from which 41 colonies were produced. The EFs for BRDs of 0.9 and 1.9 cm were about 16 and 18, respectively.

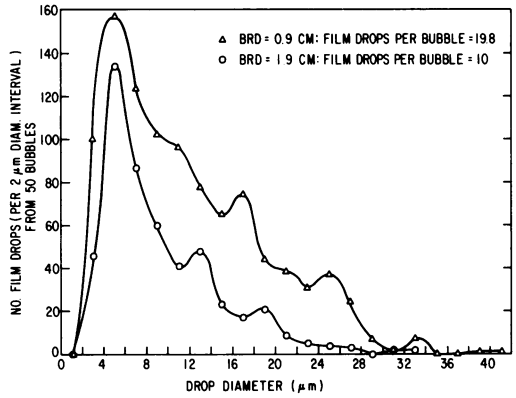

FIG. 3. Film drop size distribution obtained from 50 bursting bubbles for BRDs of 0.9 and 1.9 cm.

DISCUSSION

The change from distilled water to pond water in the *S. marcescens* suspension made little difference in the film drop EF, nor did an increase in the BRD by a factor of two. However, when the BRD went from 0.9 to 1.9 cm, very obvious changes in drop size and number occurred (Fig. 3). Although the general shape of the distribution remained the same, with a maximum production for 5-μm drops, the number of drops in each size range decreased. It is likely that this was caused by subtle differences in the position of the bubble relative to the surface at the moment of bursting. At the 0.9-cm depth, essentially all the bubbles burst instantly upon reaching the surface. By "instantly" we do not mean that they burst with no time delay after the upper part of the bubble reached the surface. To the unaided eye there appears to be no time delay, but careful observation through a low-power telescope and with good lighting shows that the buoyant force of these bubbles causes them to rise up through the surface, far above the equilibrium position shown in Fig. 1. The higher the bubble rises above the surface, the larger becomes the bubble film area. At a distance of nearly a bubble diameter above the surface, the bubble bursts and a maximum number of film drops, in this case nearly 20, are produced. Analysis of high-speed movies shows that this occurs with a time delay of a few milliseconds.

Interestingly a BRD about twice as great, 1.9 cm, produced only half as many drops. Why? Recall that at the greater depth we encountered a much higher proportion of our bubbles sticking on the surface for several seconds, and rejected them to sample only the drops produced by the bubbles which appeared to the eye to burst instantly. It is quite likely that in this case the bubbles rose up through the surface, as they always do, producing a maximum bubble film area. However, bubble bursting does not occur at this time. The inertial effects of the buoyant force are overcome by the downward-directed surface tension forces, and the bubble begins to move down to the equilibrium, steady-state position (Fig. 1a). Before it reaches that position, however, a sufficient drainage of water has occurred, thinning the film, and bursting takes place. The film area is now less than in the case when the BRD is 0.9 cm, and thus only 10 drops are produced. The time delay can be <10 ms and not detectable to the eye. These small differences in bubble position for bubbles that burst "instantly" not only affect film drop production but also cause dramatic differences in both size and number of jet drops (3, 7).

Both the number and the size of the film drops shown in Fig. 3 are compatible with what little is known of film drop distributions (3, 5, 11); however, it is likely that there are film drops of <1 μm which escaped detection by the electrostatic method (10). For several reasons, the lower cutoff for electrostatic collection is between 1 and 2 μm. Recent evidence (10) suggests a bimodal film drop distribution, with a second peak at <1 μm diameter. Bacteria are unlikely to be concentrated in these drops, but viruses may be (1). As is typical of most aerosol distributions, the highest concentration contains a small percentage of the volume. For example, in the distribution in Fig. 3 for a BRD of 0.9 cm, drops of <10 μm constitute 50% of the total number but only 4% of the volume.

When the BRD was 1.9 cm, the total volume of the 10 film drops per bubble was 9.8×10^{-9} cm^3, whereas the total volume for the 20 drops from a bubble with a BRD of 0.9 cm was about four times more, 3.9×10^{-8} cm^3. The latter is still more than 70 times less than the volume of one of the several jet drops produced by the bubble. Though these large jet drops (about 175 μm in diameter), whose EF is probably about unity (2, 4), carry nearly 10 times the number of bacteria carried by the 10 film drops (about 1 compared to 0.1), the jet drop is so large that it returns to the water almost immediately. It plays no role in the dispersal of a bacterial aerosol, except in very strong winds and rapid evaporation.

The EFs found in this work, between 10 and 20, are the first direct measurements of film drop EF. They are less than the values of 50 to 100 reported by Cipriano (Ph.D. thesis), who had to assume (we believe correctly) he was sampling film drops. The discrepancy is not hard to rationalize. Cipriano's bubbles had a much larger BRD than did ours, possibly as much as 10 times larger. One would expect, though it is not obvious in the experiment shown in Fig. 3, that film drop EF would increase with BRD in much the same way that jet drop EF does (8). Also, we must be careful in comparing experiments in which different species of bacteria are used. Cipriano used *Serratia marinorubra* in seawater. Not only do different species of bacteria give different EFs, but the same species can give different EFs depending upon the growth conditions used (2, 7, 15).

The bubbles burst at a surface that was continuously overflowing. Had the surface been stagnant, the *S. marcescens* would have concentrated in the surface microlayer (16), and the EFs probably would have been larger. The EFs in this work were produced by bacteria collected on the bubble as it rose through the water. Experiments at a stagnant surface should be done, though it is likely that a microlayer, even if well formed, would be destroyed by the breaking wave that produces the bubbles. Additionally, the drag force of the many thousands of bubbles (10) rising to the surface produces an upwelling of the water. This phenomenon, easily observed after a wave breaks, causes surface divergence which pushes radially outward any remnants of a concentrated microlayer. Thus, many of the bubbles burst at a clean surface, and the only source of a high EF in the film drops is the material scavenged by the bubbles.

Since recent work has implicated a lung-depositable aerosol produced by splashing water in the production of disease in humans (12, 17), the manner in which these pathogens are being concentrated in film drops should be understood. Further experiments in which both BRD and bubble size are increased beyond the values reported here clearly are in order. The electrostatic induction technique allows an unambiguous determination of the EF of bacteria in film drops.

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